disorders using DBS, among other sources of biological samples. In particular embodiments, the subjects are newborns and the DBS are already routinely collected as part of existing NBS procedures. In particular embodiments, the samples can include buccal swabs, peripheral blood mononuclear cells (PBMCs), or white blood cells (WBCs) collected in the clinic for follow up confirmation after presumptive positive result from NBS. In particular embodiments, the assays can predict whether a subject will develop an immune reaction to ERT and distinguish cases of enzyme pseudo deficiency from confirmed LSD patients.

[0013] The current disclosure describes peptides associated with each of the disorders that can be reliably detected and quantified using peptide immunoaffinity enrichment coupled to selected reaction monitoring mass spectrometry (immuno-SRM). The current disclosure also provides high affinity antibodies that can be used to enrich for the indicated peptides.

[0014] In particular embodiments, an antibody or antigen binding fragment thereof of the disclosure includes: a heavy chain variable (VH) domain including CDR1 of SEQ ID NO: 5, CDR2 of SEQ ID NO: 6, and CDR3 of SEQ ID NO: 7, and a light chain variable (VL) domain including CDR1 of SEQ ID NO: 8, CDR2 of SEQ ID NO: 9, and CDR3 of SEQ ID NO: 10. In particular embodiments, an antibody or antigen binding fragment thereof of the disclosure includes a VH domain as set forth in SEQ ID NO: 13 and a VL domain as set forth in SEQ ID NO: 16. In particular embodiments, an antibody or antigen binding fragment thereof of the disclosure includes: a VH domain including CDR1 of SEQ ID NO: 17, CDR2 of SEQ ID NO: 18, and CDR3 of SEQ ID NO: 19, and a VL domain including CDR1 of SEQ ID NO: 20, CDR2 of SEQ ID NO: 21, and CDR3 of SEQ ID NO: 22. In particular embodiments, an antibody or antigen binding fragment thereof of the disclosure includes a VH domain as set forth in SEQ ID NO: 25 and a VL domain as set forth in SEQ ID NO: 28. In particular embodiments, the disclosure provides assays and kits including an antibody or antigen binding fragment thereof described herein. In particular embodiments, the antibody or antigen binding fragment thereof is a recombinant antibody or antigen binding fragment thereof.

[0015] Particular embodiments include using the antibodies or antigen binding fragments thereof of the disclosure to screen for MPS I and/or Pompe Disease in newborns and also high-risk subjects. In particular embodiments, the antibodies or antigen-binding fragments thereof can be used to determine true positive cases, to eliminate pseudo deficiency, and to determine efficacy of one or more treatments in a subject being treated for MPS I and/or Pompe Disease. Particular embodiments include using the antibodies or antigen binding fragments thereof of the disclosure to detect one or more signature peptides of MPS I and/or Pompe Disease in one or more biological samples. The disclosure also provides a method for predicting whether a subject will develop an immune response to enzyme replacement therapy (ERT) for MPS I and/or Pompe Disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Some of the drawings submitted herein may be better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserves the right to present color images of the drawings in later proceedings.

[0017] FIG. **1**. Table listing protein targets and peptide sequences used for peptide immunoaffinity enrichment coupled to selected reaction monitoring mass spectrometry (immuno-SRM-MS) for Mucopolysaccharidosis Type I (MPS I; Hurler Syndrome) and Pompe Disease. Total mass, parent ion mass, daughter y-ion masses, and daughter b-ion masses are also shown.

[0018] FIG. **2**. Schematic illustrating the process of immuno-SRM-MS.

[0019] FIGS. **3**A-**3**D. A study of 11 MPS I disease patients (9 pre-treatment patients including 2 with post-treatment samples: 2 attenuated form; 4 severe form; and 3 unknown form. Two post-treatment patients) (ERT: enzyme replacement therapy; BMT: bone marrow transplant; LOD: lower limit of detection). (FIG. **3**A) IDUA 218 peptide biomarker level in 100 normal controls and 11 MPS I patients (dotted line represents the cutoff for IDUA 218). (FIG. **3**B) enlarged version of FIG. **3**A with the focus from 0 to 10 pmol/L (dashed line represents the LOD of IDUA 218). (FIG. **3**C) IDUA 462 peptide biomarker level in 100 normal controls and 11 MPS I patients (dotted line represents the cutoff for IDUA 462). (FIG. **3**D) enlarged version of FIG. **3**C with the focus from 0 to 5 pmol/L (dashed line represents the LOD of IDUA 462).

[0020] FIGS. **4**A, **4**B. Linear response of IDUA peptides when an internal standard was spiked into the dried blood spot (DBS) matrix (the dotted line represents the lowest level of peptide found in normal cohort).

[0021] FIGS. **5**A, **5**B. IDUA peptide concentrations in DBS and peripheral blood mononuclear cell (PBMC) samples. (DBS: five 3 mm punches; PBMC: 250 μg of protein).

[0022] FIGS. **6**A, **6**B. IDUA peptide concentrations in DBS and buccal swab samples. (DBS: five 3 mm punches).

[0023] FIGS. **7**A, **7**B. Comparison of IDUA concentrations among 100 normal controls (NC), 9 MPS I patients (MPS I pt), and 4 MPS I pseudo deficient cases (MPS I Pseudo) for IDUA 218 peptide biomarker (FIG. **7**A) and IDUA 462 peptide biomarker (FIG. **7**B).

[0024] FIGS. **8**A, **8**B. Multiple reaction monitoring (MRM) traces for GAA peptides from purified peptides (left) and DBS samples (right) after peptide capture by sera antibodies from immunized rabbits. (FIG. **8**A) GAA 332 MRM traces; (FIG. **8**B) GAA 855 MRM traces.

[0025] FIG. **9**. Endogenous multiple reaction monitoring (MRM) traces for GAA 855 from PBMC sample after peptide capture by supernatant antibodies from isolated plasma cells.

[0026] FIGS. **10**A, **10**B. Multiple reaction monitoring (MRM) traces for GAA peptides from purified peptides (I), DBS samples (II), and buccal swab samples (III) after peptide capture by sera antibodies from immunized rabbits. (FIG. **10**A) GAA 155 MRM traces, TTPTFFPK (SEQ ID NO: 3), parent ion mass 469.7527++; (FIG. **10**B) GAA 376 MRM traces, WGYSSTAITR (SEQ ID NO: 5), parent ion mass 571.2855++.

[0027] FIG. **11**. Comparison of GAA concentrations in DBS among three normal controls (NC1, NC2, NC3), three true positive Pompe patients (PD1, PD2, PD3), and two pseudo-deficient cases (Pseudo 1 and Pseudo 2) for GAA 376 peptide biomarker (WGYSSTAITR (SEQ ID NO: 5), parent ion mass 571.2855++).